A NEW SPECIES OF *PEROPHORA* (ASCIDIACEA) FROM THE WESTERN ATLANTIC, INCLUDING OBSERVATIONS ON MUSCLE ACTION IN RELATED SPECIES

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ABSTRACT

Perophora regina n. sp. is described from Twin Cays, Belize, Central America. It differs from other western Atlantic species of *Perophora* in colony form, structure of the testis and mantle musculature. The action of the mantle musculature in the three Caribbean species of *Perophora* is compared.

The Perophoridae Giard, 1872, is a family of phlebobranch ascidians which form colonies of small replicating zooids connected to one another by a system of stolons which ramify and anastomose over the substratum; new zooids are formed as buds arising on the stolons. Only two genera are recognized in the family. The genus *Perophora* Wiegmann, 1835, is characterized by the small size (2–10 mm) and simplicity of the zooids, which usually have only four and never more than eight rows of stigmata in the branchial sac. The genus *Ecteinascidia* Herdman, 1880, differs only in the larger size of the zooids (5–25 mm) and greater number of rows of stigmata, usually in excess of 10, in the branchial sac. Both genera are characteristic of warm seas throughout the world, and *Perophora* extends into temperate seas.

In the western Atlantic region two species of *Perophora* have been recognized up to the present time. *Perophora viridis* Verill, 1871, has four rows of stigmata, a rounded stomach and four or five lobes to the testis. *Perophora formosana* (Oka, 1931) has five rows of stigmata, the fifth arising by division of the most anterior of the original four rows; it has an oval stomach and a single testis lobe. *Perophora bermudensis* Berrill, 1932, is now considered to be a synonym of *P. formosana* (C. Monniot, 1983). In addition to the characters mentioned above there are differences in the musculature of *P. viridis* and *P. formosana*; these are further discussed below. *P. viridis* occurs from Cape Cod, U.S.A., to the Guyana Shelf in Northern South America (Van Name, 1945; Millar, 1978). *P. formosana* ranges from Bermuda to Florida and throughout the West Indies (Van Name, 1945; Monniot, 1983; Goodbody, 1984a; 1984b).

In the course of studies of the ascidians of the Barrier Reef in Belize, Central America, we have found another species of *Perophora*, which is characterized by its size, colony growth form, structure of the testis and strikingly different arrangement of mantle muscles. We propose that this handsome ascidian should be named *Perophora regina*.

Perophora regina new species
Figures 1–6

Colony Form.—Colonies have a mass of ramifying and anastomosing stolons creeping over the substratum; each zooid is borne on a vertical stalk arising from this basal mass. Colonies vary in size from a few scattered zooids to many hundreds of closely packed zooids. The largest colony recorded in the field extended 45 cm along the length of a root and contained more than 600 zooids.
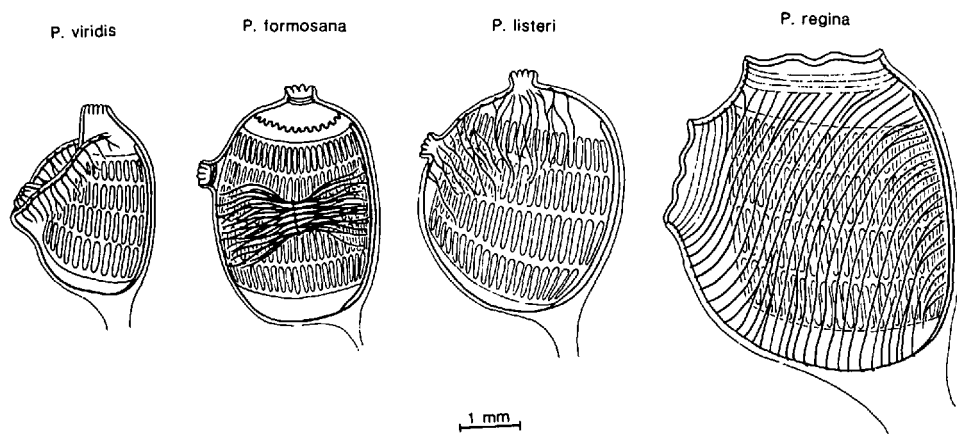
Figure 1.   The arrangement of mantle musculature in relation to the siphons in four Atlantic species of *Perophora*.

The stalk at the posterior of each zooid comes off at an angle to the endostyle and not in line with it, and the orientation of zooids in the colony is such that the neural complex faces outwards, the branchial sac is at an angle of approximately 45° to the substratum and the atrial aperture faces out towards the periphery of the colony (see Fig. 4A). There are many variations on this general arrangement depending on the age and position of a zooid within the colony. The orientation of zooids in the colony does not alter the fact that in respect to the anterior posterior axis of the zooid the oral siphon is terminal and the atrial siphon is dorsal.

Zooid Structure. — Zooids range from 4–6 mm in length and vary in shape because of the effect crowding has on growth form; many are vase-shaped with a narrow base leading into the vertical stolon or stalk, which connects the zooid to the basal mass of stolons. The body is translucent grey/green in color, but the neural complex is bright orange and bounded on either side by a broad patch of white pigment. Living animals are often in constant motion due to contraction and expansion of the mantle musculature, giving a "dancing" effect to the whole colony; this is more frequent in those parts of the habitat where there is a high concentration of suspended matter in the water.

The large oral siphon is terminal and usually has a delicate fringe of lobes, usually eight in number but sometimes more; in living animals the lobes are often indistinct and the siphon margin appears smooth-lipped. The atrial siphon is similar but not so large, set at an angle antero-dorsally with a large atrial space between it and the branchial sac. The mantle musculature is principally oblique, extending from the base of the oral siphon to the posterior end of the zooid; the muscle fibers are large, prominent and powerful. Dorsally these muscle fibers grade into the circular muscle system of the atrial siphon, and ventrally some finer muscles arise along the line of the endostyle and run obliquely toward the alimentary canal. Both siphons have fine circular muscles but the longitudinal musculature is indistinct (Fig. 1).

The branchial sac has four rows of stigmata, each with 35 to 40 stigmata. There are 11 to 12 prominent papillae on each transverse bar; each papilla gives rise to an anterior and a posterior branch within the branchial sac but these do not link with those from adjacent transverse bars to form any inner longitudinal bar. There
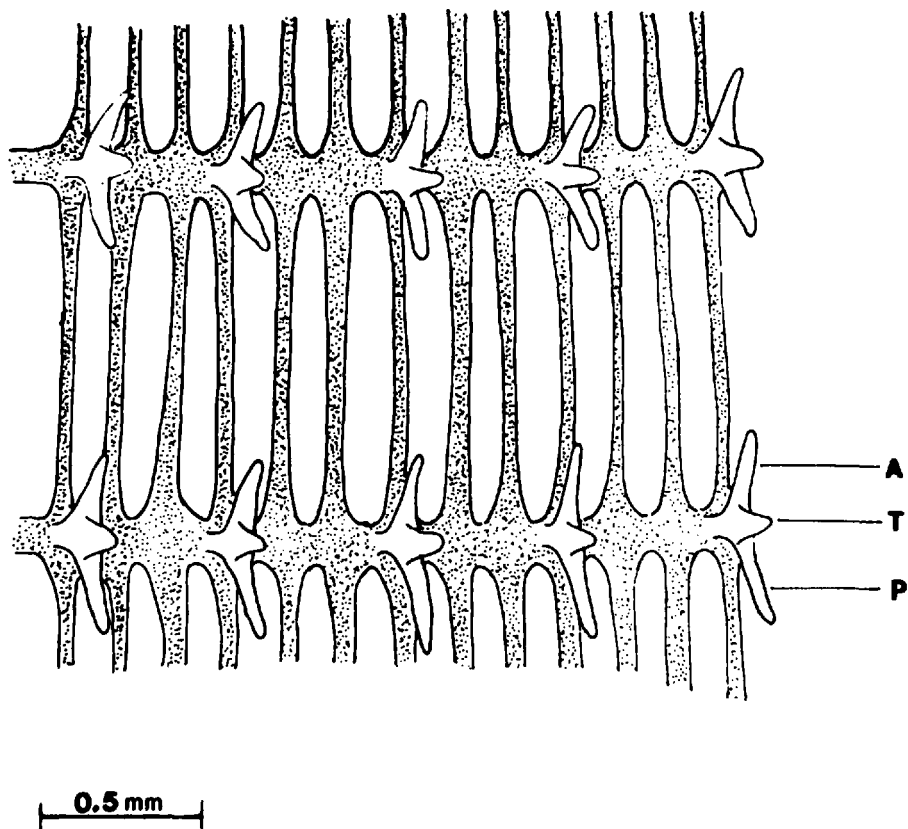
Figure 2.　A portion of the inner wall of the branchial sac in *P. regina* showing the trifid arrangement of the papillae: A, Anterior process; P, Posterior process; T, Secondary or inner papilla.

is a prominent secondary papilla arising between the anterior and posterior arms of the primary papilla so that the entire structure is trifid (Fig. 2). The dorsal lamina is a smooth membrane with three stout languets, one placed at each transverse vessel. The number of branchial tentacles appears usually to be about 20 to 24 of slightly varying length.

The alimentary canal forms a loop enclosing the gonads in the posterior part of the zooid (Figs. 3 and 4). The esophagus has a wide funnel and leads to a globular smooth-walled stomach. Shortly after leaving the stomach the intestine is narrowly constricted for a short length and then expands again, curving to the anus, which is normally smooth-lipped, though sometimes faintly lobulated.

The gonads are on the left side in the loop of the alimentary canal, the testis slightly ventral to the ovary. The testis comprises 12 to 15 separate lobes each one connected by a separate duct to a common sperm duct which emerges from within the center of the lobular mass. The system of individual ducts is not visible from outside the testis mass but has been verified by serial sectioning of the gonad. In early development the testis lobes appear as clearly separate rounded masses of spermatogonia (Fig. 3A); as these enlarge they press close to one another, lose their rounded shape and become irregular in outline (Fig. 3B). The ovary develops later than the testis and occupies a position to the left of the base of the sperm duct just dorsal to the testis. As the eggs mature and enlarge they occupy a position
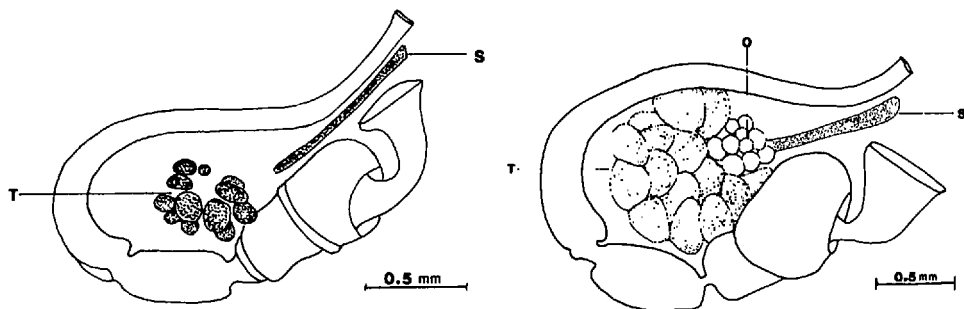
Figure 3A.   (Left) An early stage in the development of the testis in *P. regina* showing the individual development of testis lobules. The ovary and oviduct are not yet visible: T, Testis lobules; S, Sperm duct.

Figure 3B.   (Right) A later stage in gonad development of *P. regina*. The testis lobules are now full and the ovary appears as a cluster of small eggs at the base of the sperm duct: O, Ovary; S, Sperm duct; T, Testis.

more to the left of the testis itself (Fig. 4A). Usually only 6 to 12 eggs develop in the ovary at any one time. The slender oviduct extends dorsally from the ovary parallel to the sperm duct but just before reaching the tip of the sperm duct it turns through a right angle, crosses over the dorsal lamina of the branchial sac and opens on the right side of the animal behind the last row of stigmata close to the dorsal lamina (Fig. 4). This arrangement of the oviduct is similar to that found in other members of the Family (Berrill, 1950; Mukai et al., 1983; Kott, 1985).

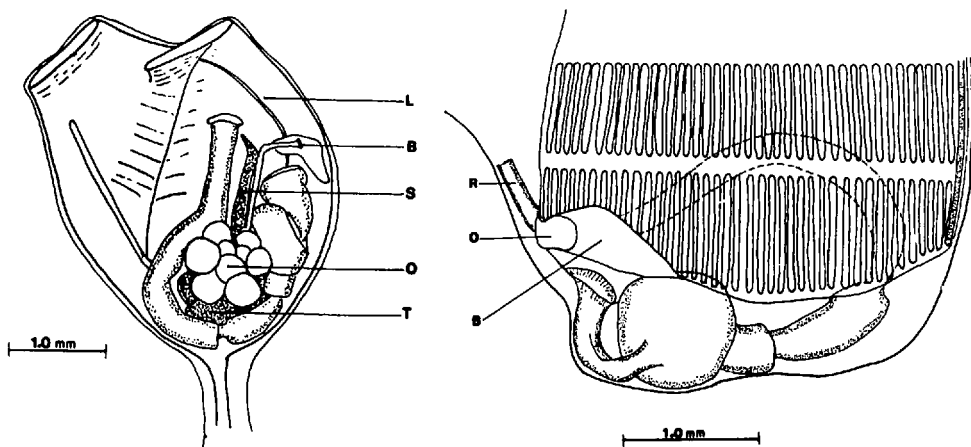The embryos develop in a brood pouch which seems to develop from the lining

Figure 4A.   (Left) The female reproductive system of *P. regina*. The mature ovary lies to the left of the testis and the oviduct is seen to turn over the dorsal lamina to enter a brood pouch on the right side of the atrial cavity. Note the orientation of the zooid on the stalk so that the branchial sac is at an angle of about 45°. The diagonal fold in the mantle wall is the result of strong muscular contraction at fixation: B, Brood pouch; L, Dorsal Lamina; O, Ovary; S, Sperm duct; T, Testis.

Figure 4B.   (Right) The early development of the brood pouch, in *P. regina,* as a derivative of the atrial epithelium surrounding the tip of the oviduct. The sperm duct has been omitted for clarity: B, Brood pouch; O, Oviduct; R, Rectum.
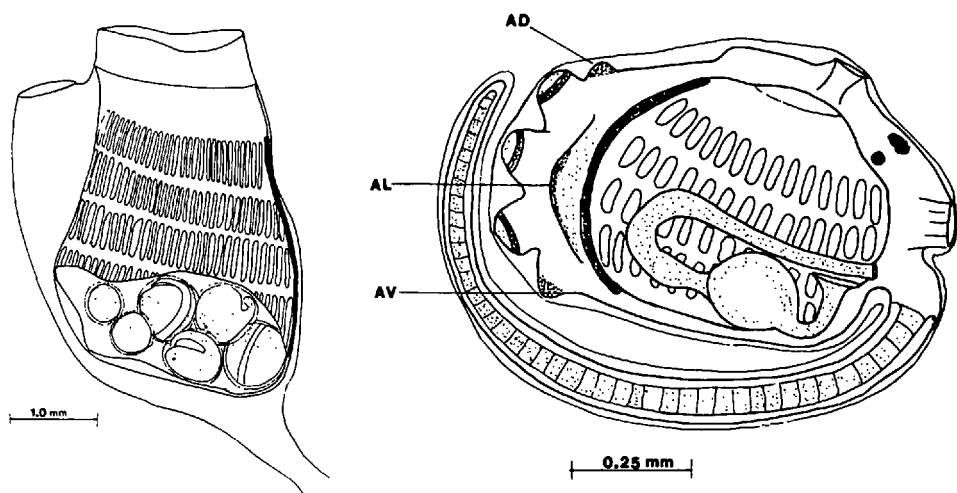
Figure 5A.   (Left) The mature brood pouch of *P. regina* with developing embryos.

Figure 5B.   (Right) The larva of *P. regina*: AD; Dorsal ampulla; AL, Lateral ampulla; AV, Ventral ampulla.

of the peribranchial cavity on the right side, posteriorly. It first appears as a small slit-like cavity surrounding the tip of the oviduct at the time that eggs are beginning to ripen in the ovary (Fig. 4B). Eggs are discharged directly into the brood pouch, which enlarges as the number of developing embryos increases. When the pouch is full it lies in the posterior end of the right side of the peribranchial cavity pressing against the last row of stigmata (Fig. 5A). The largest embryos are at the distal or ventral end of the pouch and the smallest (or youngest) at the proximal or dorsal end. Once it is full the pouch appears to close off as a separate saccular structure, and the oviduct separates from the pouch. The mechanism by which larvae are released from this pouch has not been observed.

Larva (Fig. 5B). — The larva, which reaches a size of 1 mm trunk length, has already developed siphons, branchial sac and gut. There is a prominent sensory vesicle containing ocellus and otolith, and three attachment papillae are arranged in a vertical row at the anterior end. There are four ampullae, one dorsal, one ventral, and on each side a lateral one.

Type Locality. — The species was first found in the mangrove channels at Twin Cays, Belize, Central America (16°49′N, 88°06′W), where it grows on the hanging roots of the mangrove *Rhizophora mangle* (L.). It is particularly abundant where there is a regular flow of water, as at the northern entrance to the main channel, or in the smaller channels of Turtle Cove and Hidden Creek where the tidal flow is strong. Although it may be found anywhere along the length of a root and in association with other sessile organisms, it is very commonly found at the apical growing tip of the root where competition from other organisms is at a minimum. Small colonies have also been found at the Blue Ground Range a few miles south of Twin Cays.

Specimens. — The Holotype and three Paratypes were collected from the North Channel in Twin Cays, Belize, Central America, in approximately 1 m of water. The colonies grow over pieces of mangrove root some of which have been cut in
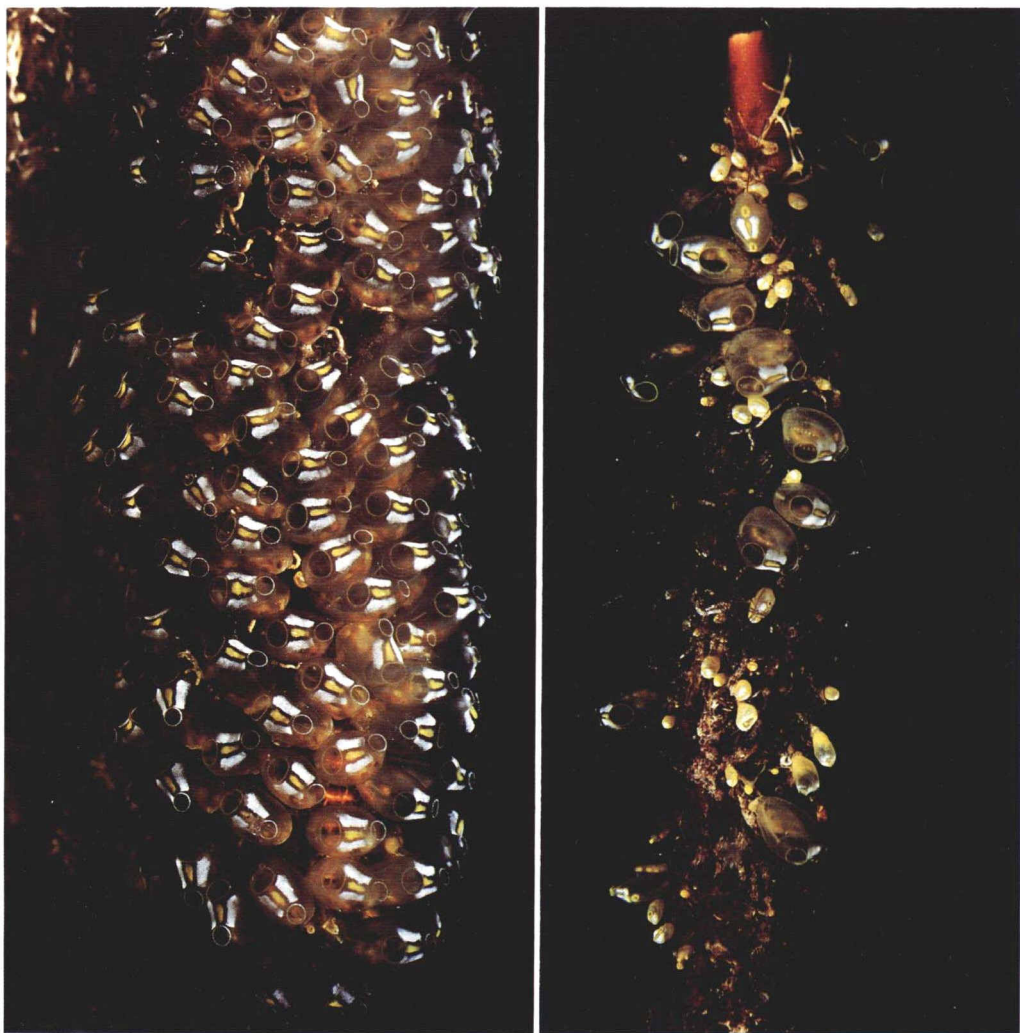
Figure 6.   On the left *Perophora regina* n. sp., a large colony growing on a *Rhizophora* root. Individual zooids in the colony are clearly defined by the two siphons between which is seen the orange neural complex bounded on either side by a patch of white pigment. Twin Cays, Belize, 9 June 1985. On the right a young colony growing on the tip of a *Rhizophora* root showing creeping stolons, buds in various stages of development and fully grown zooids. Twin Cays, Belize, 6 May 1986.

two for storage purposes. All colonies were narcotised in menthol, fixed in form-aldehyde, stored temporarily in 4% formaldehyde and have been transferred to alcohol for permanent storage. They are deposited in the National Museum of Natural History, Washington D.C., and are designated as follows:

HOLOTYPE. Colony containing in excess of 150 zooids collected 30 March 1981 (U.S.N.M. No. 16288).

PARATYPES. (i) Colony of approximately 75 zooids collected 30 March 1981 (U.S.N.M. No. 16289); (ii) Colony of approximately 100 zooids collected 30 March 1981 (U.S.N.M. No. 16290); (iii) Colony of approximately 150 zooids collected 29 February 1984 (U.S.N.M. No. 16291).

DISCUSSION

Perophora regina is characterized by the compact form of the colony, the prominent and complete oblique mantle musculature, the large number of testis lobes (up to 15), and the distinctive coloration of the neural complex and adjacent mantle area.

The species seems most closely related to *Perophora modificata,* recently described from Australia by Kott (1985). Both species have large zooids arising from a tangled mat of stolons and are similar in the orientation of the zooid on the stalk, large number of long stigmata and large number of testis lobes. *P. modificata* differs from *P. regina* in having a thickened test, longitudinal rather than oblique muscles, and in the possession of a central vascularized extension of the body with paired stolonic vessels at the posterior end. The trifid nature of the branchial papillae in *P. regina* appears to be unique amongst the members of the genus. *P. regina* differs from the other western Atlantic species, *P. formosana* and *P. viridis,* in its size, large number of testis lobes and in the form and extent of its mantle musculature.

The functional purpose of the orientation of the zooids in the colony in both *P. regina* and *P. modificata,* is probably related to crowding and the necessity of ensuring that the atrial aperture can discharge its contents well clear of the colony. As a consequence of the angled orientation the neural complex is fully exposed to the exterior and the development of pigment in this region is probably protective. *P. regina* is a shallow water species and in certain environs is fully exposed to bright illumination. The pigment is most well developed in colonies living in such situations and is less well developed in those living in shaded places. Goodbody (unpubl. obs.) has observed a similar development of pigment around the neural complex of *Corella minuta* if it is removed from its normal shaded environment to a brightly illuminated position. Kott (1985) does not mention pigmentation of this sort in *P. modificata* which is not unexpected as this species is found in deeper water (17 to 27 m).

The differences in mantle musculature found amongst members of the genus are also of functional significance as is illustrated in the three western Atlantic species. In *P. viridis* there is a flat plate of tissue between the two siphons, which we are calling the siphonal field (Fig. 1). The mantle musculature is largely confined to a system of radially arranged fibers in this field, which also extend a very short way onto the right and left sides of the mantle wall. The effect of this arrangement is to make the entire siphonal field act as a type of diaphragm which pulsates in and out at the anterior end of the animal. Branchial pressure must be controlled by this diaphragm in much the same way that the laterally placed musculature of *Ascidia nigra* controls pressure in that species (Goodbody and Trueman, 1969). When the siphonal field contracts strongly the atrial siphon is pulled down into a deep fold of the test; there is only limited withdrawal of the branchial siphon. The antero-lateral fibers of the mantle wall can contract independently of those in the siphonal field; this does not appear to exercise any degree of fine control of the volume of the mantle cavity, but does cause a slight folding in its lateral wall.

In *P. formosana* there is no siphonal field, and the two siphons are set almost at right angles to one another: the oral siphon anterior, the atrial dorsal. Each siphon has a system of fine delicate muscle fibers of its own which appear to be concerned only with siphonal control. Control of the mantle cavity is exercised by a system of fine delicate muscle fibers running transversely across the dorsal side of the animal behind the atrial siphon. These muscles have a lateral node on

either side and are anchored ventrally lateral to the endostyle. The muscles thus form a girdle around the zooid at the level of the third stigmatal row. This system of muscles provides control of mantle activity by causing a "waisting" or "pouching" of the dorso-lateral wall of the mantle similar to the effect created by a person pulling in their cheek muscles. This dorso-lateral contraction takes place over a large atrial space between the atrial siphon and the branchial sac. Observation of living animals suggests that this is a more delicate control of mantle activity than is exercised by the siphonal field "diaphragm" of *P. viridis* and probably affects the feeding and ecology of the two species differently. In Figure 1 we have also illustrated the muscle condition in the European species *Perophora listeri* for comparison with *P. viridis* and *P. formosana,* but we have not had an opportunity to see this species alive.

Perophora regina exercises control over the mantle in a completely different manner which resembles the activity seen in living *Diplosoma.* The strong muscles of *P. regina* cause contraction of the whole branchial axis and may throw the body into one or more folds. In contrast to the somewhat delicate control of mantle activity seen in the other species of *Perophora,* especially in *P. formosana, P. regina* engages in forceful pulsations of the body, retracting and expanding rapidly in response to extraneous stimuli. Continuous activity of this sort gives rise to the dancing effect referred to earlier. This type of muscle action probably makes it possible for the animal to colonize and survive in the strong currents found in its normal habitat.

The structural features of the reproductive system of *Perophora regina* are similar to those found in other members of the genus, as described by Mukai et al (1983). The arrangement of the oviduct and the brood pouch is similar to that in *Perophora formosana* and *P. japonica.* The closed distal end of the pouch resembles the condition found in *P. sagamiensis,* but in that species there is considerable degeneration of adult structures following filling of the brood pouch, a condition we have not demonstrated in *P. regina.* It is to be noted that the brood pouch is not an expansion of the oviduct as is suggested by Kott (1985) for *P. modificata,* but is an independent development arising from the wall of the peribranchial or atrial cavity (Mukai et al., 1983).

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